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THREE NEW ω-CYCLOHEPTYL FATTY ACIDS FROM *ALICYCLOBACILLUS CYCLOHEPTANICUS* AND THEIR BIOSYNTHETIC INTERRELATIONSHIPS

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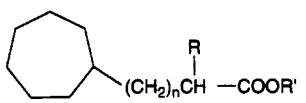
ABSTRACT.—Three minor ω-cycloheptyl fatty acids from *Alicyclobacillus cycloheptanicus* have been identified as ω-cycloheptylnonanoate, -decanoate, and -α-hydroxytridecanoate. The biosynthetic interrelations of these cyclic fatty acids have been studied. ^{13}C-Labeled ω-cycloheptylundecanoate and -α-hydroxyundecanoate were converted to ω-cycloheptyldecanoate, which is one carbon shorter in length. α-Hydroxylation of ω-cycloheptylundecanoate was also observed, but not chain extension to ω-cycloheptyltridecanoate.

The fatty acid mixture from the lipids of the thermoacidophile *Alicyclobacillus cycloheptanicus* is dominated by ω-cycloheptyl fatty acids, which are unique to this organism (1–3). ω-Cycloheptyl fatty acids, such as the homologous ω-cyclohexyl fatty acids of *A. acidocaldarius* and *A. acidoterristris*, are suggested to enable the organism to grow in acidic, hot media by providing a more dense cell membrane (1,4).



	n	*R*		
1	9	H		
2	11	H		
3	9	OH		
4	7	H	**a**	R′=H
5	8	H	**b**	R′=CH_3
6	11	OH		

In the course of studies on the biosynthetic pathway of formation of the biologically novel structural feature of the saturated cycloheptane ring (5,6), we identified three minor ω-cycloheptyl fatty acids in the membrane lipids of this organism. Their structure as well as their biosynthetic relations to the other known ω-cycloheptyl fatty acids are presented in this paper.

ω-Cycloheptylundecanoic acid [**1**], ω-cycloheptyltridecanoic acid [**2**], and ω-cycloheptyl-α-hydroxyundecanoic acid [**3**] comprise nearly 80% of the total fatty acids obtained upon saponification of *A. cycloheptanicus* total lipids (1). The remaining fatty acids were reported to be a mixture of branched- and straight-chain fatty acids. These minor components were analyzed by gc-ms (Figure 1) as their methyl esters, following CH_2N_2 treatment of the fatty acid mixture, to probe for the occurrence of any additional ω-cycloheptyl fatty acids.

Three of the minor fatty acid methyl esters (**4**, **5**, and **6**) strongly fragmented to *m/z* 97 and M^+-97, which is characteristic of ω-cycloheptyl fatty acid methyl esters (3). These intense peaks result from the cleavage of the cycloheptane ring from the fatty acid chain. The mass spectra of compounds **4** and **5** also contain large peaks at *m/z* 74 and 87, indicative of a fatty acid methyl ester without substituents at the α-position (7). The mass spectra of these methyl esters are consistent with the structures for methyl ω-cycloheptylnonanoate [**4b**] (M^+ 268) and methyl ω-cycloheptyldecanoate [**5b**] (M^+ 282). The third compound [**6b**] (M^+ 340) gave a spectrum similar to that of **3b** (2) with typical α-hydroxy fatty

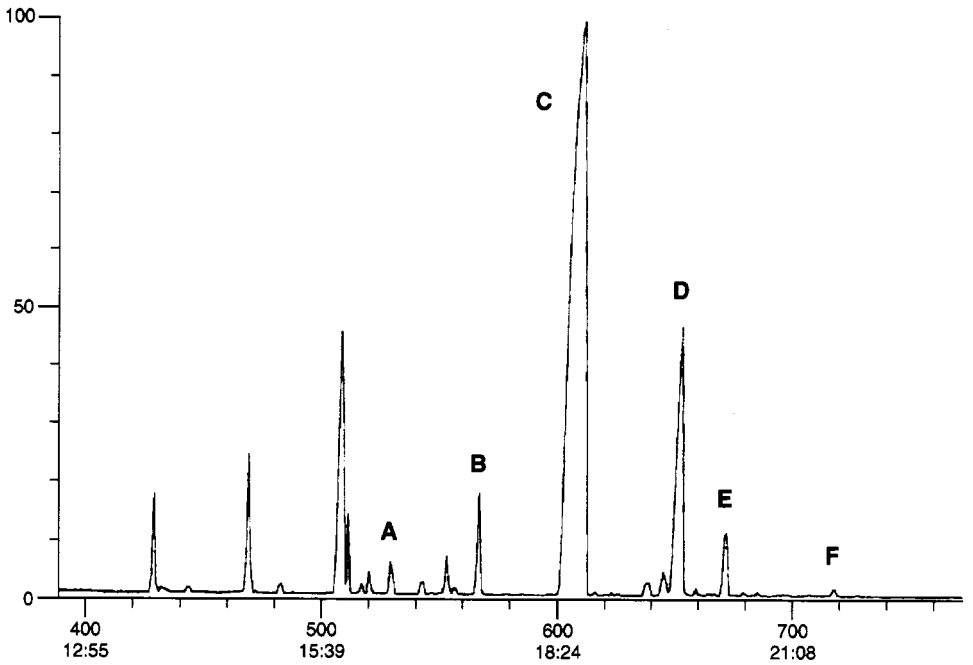


FIGURE 1. Total ion chromatogram of *A. cycloheptanicus* fatty acid methyl esters. Peaks A, B, C, D, E, and F correlate to compounds **4b**, **5b**, **1b**, **3b**, **2b**, and **6b**, respectively.

acid fragmentation to *m/z* 90 $[CH_3OC(OH)=CHOH]^+$ and 103 $[CH_3OC(O)CH(OH)CH_2]^+$ (8). The proposed structure for **6b** is therefore methyl ω-cycloheptyl-α-hydroxytridecanoate, the α-hydroxy derivative corresponding to **2b**.[1]

The results of numerous feeding experiments have demonstrated that cycloheptanecarboxylic acid (probably as its CoA thioester) serves as the starter unit for ω-cycloheptyl fatty acid biosynthesis in *A. cycloheptanicus* (5,6). In the case of **1**, five acetate units are added to cycloheptanecarboxylic acid to form the fatty acid chain. However, the acetate-derived portion of the fatty acid chain of **4** contains an odd number of carbons, which correlates to the loss of half of an acetate unit. Fatty acid **4** is probably formed from **1** through α-hydroxylation, dehydrogenation, and subsequent oxidative decarboxylation This transformation has precedence in *Tetrahymena pyriformis*; exogenously added α-hydroxypalmitic acid yields the non-hydroxy fatty acid, pentadecanoic acid, which is one carbon atom shorter in length (9).

The interrelationships of the cyclic fatty acids in *A. cycloheptanicus* were examined by feeding ^{13}C-labeled **1** and **3**, which had been previously obtained from a feeding experiment with [8-^{13}C]cycloheptanecarboxylic acid (5,6). [11-^{13}C]-**1** (1.0 mg, 50% ^{13}C enrichment) and [11-^{13}C]-**3** (0.3 mg, 50% ^{13}C enrichment) phenacyl esters, isolated by reversed-phase hplc (5,6), were hydrolyzed with 10 equivalents of NaOH at room temperature overnight. The basic solutions were then added by sterile filtration to separate 100-ml cultures of *A. cycloheptanicus*. After 24 h of growth, the biomass from each culture was saponified, the fatty acids were extracted and analyzed as methyl esters by gc-ms (Table 1).

The hydroxyundecanoic acid [11-

[1]Mass spectra of all six ω-cycloheptyl fatty acid methyl esters are presented by Moore (5).

TABLE 1. Mass Spectral Analysis of Compounds **1b**, **2b**, and **3b** from Feeding Experiments with [11-^{13}C]-**1a** and -**3a**.

Precursor	*m/z* (Rel. int.)									
	Product [**1b**]				Product [**3b**]			Product [**5b**]		
	296	297	298	299	312	313	314	282	283	284
Unlabeled control	100	23.8	3.1	0.3	100	22.2	3.1	100	21.7	3.4
[11-^{13}C]-**1a**	100	26.1	3.6	0.4	100	23.9	2.9	100	26.6	2.9
[11-^{13}C]-**3a**	100	22.3	2.8	0.3	100	22.4	3.0	100	34.6	6.1

^{13}C]-**3** underwent α-dehydrogenation and oxidative decarboxylation, as the resultant decanoic acid **5** was 11.4% enriched. This process appears to be efficient; recovered **3** was not significantly labeled. Likewise, the undecanoic acid [11-^{13}C]-**1** was converted to **5** (4.7%), presumably through the hydroxyundecanoic acid **3**, which was also slightly enriched (1.7%). However, the ω-cycloheptanetridecanoic acid from the same experiment showed no significant enrichment. The results also suggest that, as one would expect, these transformations take place at the level of the free acids which are not in complete equilibrium with the corresponding fatty acid moieties in the lipids.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Gc-ms was carried out on either a Kratos Profile mass spectrometer or a VG MD 800 quadrupole mass spectrometer. *Alicyclobacillus cycloheptanicus* (formerly *Bacillus cycloheptanicus*) (10) was obtained from the Deutsche Sammlung für Mikroorganismen (DSM 4006). Ingredients for fermentations were from Difco and Sigma. [11-^{13}C]-**1** and -**3** had been prepared previously (5,6).

GC-MS ANALYSIS OF *A. CYCLOHEPTANICUS* FATTY ACID METHYL ESTERS.—Fermentation of *A. cycloheptanicus* and fatty acid isolation and esterification are described by Moore and co-workers (5,6). Gas chromatography was carried out on a HP-5 (cross linked 5% Ph Me silicone) column (25 m×0.2 mm i.d., 0.33 μm film thickness). The initial column temperature was 100°, and the temperature of the injector and detector was 250°. After 2 min, the column temperature was raised at 10°/min to a final temperature of 300°. Helium was used as the carrier gas. Mass spectrometry was performed on a Kratos Profile mass spectrometer using electron-impact ionization. ω-Cycloheptyl fatty acid methyl esters eluted in the following order: **4b** (*R*, 16.5 min, 1% of the total fatty acid mixture), **5b** (*R*, 17.5 min, 2%), **1b** (*R*, 18.7 min, 61%), **3b** (*R*, 19.9 min, 12%), **2b** (*R*, 20.4 min, 2%), **6b** (*R*, 22.0 min, 0.3%).

Methyl ω-cycloheptylundecanoate [**1b**].—Gc-ms *m/z* 296 (M^+, 19), 253 (5), 200 [$(M-C_7H_{12})^+$, 23], 171 (2), 157 (9), 143 (22), 129 (8), 111 (12), 97 [$(C_7H_{13})^+$, 68], 87 (93), 74 [$(C_3H_6O_2)^+$, 100].

Methyl ω-cycloheptyltridecanoate [**2b**].—Gc-ms *m/z* 324 (M^+, 28), 281 (4), 228 [$(M-C_7H_{12})^+$, 15], 185 (6), 157 (2), 143 (18), 129 (7), 111 (16), 97 [$(C_7H_{13})^+$, 70], 87 (76), 74 [$(C_3H_6O_2)^+$, 100].

Methyl ω-cycloheptyl-α-hydroxyundecanoate [**3b**].—Gc-ms *m/z* 312 (M^+, 71), 253 [$(M-CO_2CH_3)^+$, 31], 235 (3), 216 (15), 123 (14), 111 (26), 103 (16), 97 [$(C_7H_{13})^+$, 100], 90 (33).

Methyl ω-cycloheptylnonanoate [**4b**].—Gc-ms *m/z* 268 (M^+, 15), 225 (4), 172 [$(M-C_7H_{12})^+$, 13], 143 (17), 129 (13), 97 [$(C_7H_{13})^+$, 48], 87 (79), 74 [$(C_3H_6O_2)^+$, 100].

Methyl ω-cycloheptyldecanoate [**5b**].—Gc-ms *m/z* 282 (M^+, 26), 239 (5), 186 [$(M-C_7H_{12})^+$, 20], 157 (3), 143 (17), 129 (8), 97 [$(C_7H_{13})^+$, 59], 87 (90), 74 [$(C_3H_6O_2)^+$, 100].

Methyl ω-cycloheptyl-α-hydroxytridecanoate [**6b**].—Gc-ms *m/z* 340 (M^+, 40), 281 [$(M-CO_2CH_3)^+$, 14], 244 (6), 111 (30), 103 (11), 97 [$(C_7H_{13})^+$, 100], 90 (18).

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